

— Novel Ideas and Pilot Projects —

EFFICIENCY IN POLLEN FORAGING BY HONEY BEES: TIME, MOTION, AND POLLEN DEPLETION ON FLOWERS OF *SISYRINCHIUM PALMIFOLIUM* (ASPARAGALES: IRIDACEAE)

D. P. Silva*¹, J. Moisan-De-Serres², D. C. Souza³, S. B. Hilgert-Moreira⁴, M. Z. Fernandes⁴, P. G. Kevan⁵, B. M. Freitas⁶

¹Programa de Pós-Graduação em Ecologia e Evolução, Departamento de Ecologia, ICB, Universidade Federal de Goiás, Rodovia Goiânia-Nerópolis, Km 5, Campus II, Setor Itatiaia, CEP: 74001-970, Goiânia – GO, Brazil

²Programme de Biologie Végétale, Département de Phytologie, Université Laval, Québec, Canada

³Universidade Federal do Piauí, Centro de Ciências Agrárias, Departamento de Zootecnia, Campus Agrícola da Socoço

⁴Laboratório Ecologia de Abelhas, Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, Rio Grande do Sul, Brazil

⁵The Canadian Pollination Initiative, School of Environmental Sciences, University of Guelph, Guelph, Ontario N1G 2W1, Canada

⁶Universidade Federal da Ceará, Centro de Ciências Agrárias, Departamento de Zootecnia, CEP: 60.021-970, Fortaleza, Ceará, Brazil

Abstract—Honey bees depend on flower resources (nectar and pollen) to supply individual and colony needs. Although behavioural studies already assessed optimum foraging patterns of bumblebees, honey bees foraging behavioural patterns have been poorly assessed. We used *Sysirinchium palmifolium* L. (Iridaceae), a low-growing, abundant and anthophilous grassland flower to test the hypotheses that *Apis mellifera* workers would i) spend more time, ii) visit a greater number of flowers, and iii) travel greater distances within patches of *S. palmifolium* which were newly opened or not been visited by other pollinators when compared to foraging on patches that were available to pollinators during its whole blooming period (only one day). In two different sunny days, we measured bee activities in an area opened for visitation during the whole anthesis (OP plot treatment) and another opened for visitation only half of anthesis (CL plot treatment). We observed bees spending more time, visiting more flowers and travelling more in *S. palmifolium* CL treatment than the OP plot treatment. Previous studies already showed bees alter their foraging behaviour in the lack of resources. Honey bees are able to remember the period of the day when resources are usually the higher, they probably detect the most promising period to gather resources on *S. palmifolium* flowers. Since *A. mellifera* is a pollinator with a wide-distribution and is considered an important cause of changes on native pollinator communities, we support additional studies evaluating its foraging behaviours to better understand how it explores flower resources.

Keywords: *Apis mellifera*; honeybees; optimum foraging; pollen availability; *Sysirinchium palmifolium*

INTRODUCTION

Much of a worker honeybee's life consists of foraging for resources, especially nectar and pollen, to supply food for the colony and satisfy the individual forager's needs. Although there have been numerous studies in optimal foraging ecology and foraging patterns of bumblebees gathering floral nectar (Benham 1969; Pyke 1978; Waddington & Holden 1979; Zimmerman 1981, 1983; Kevan 1990; Dukas & Real 1993; Goulson 1999), fewer studies have been made on the foraging patterns of individual bumblebees foraging for pollen (Zimmerman 1982, 1983; Galen & Plowright 1985; Robertson et al. 1999) or on honey bees working at flowers for either resource (Marden & Waddington 1981; Wells & Wells 1983, 1984), especially pollen. The botanical consequences of pollinator foragers' movements are important in plant

population genetics, genetic neighbourhood sizes, and outcrossing rates in both natural vegetation (Beattie 1976) and crops (Cresswell 1997; Kron et al. 2001a, b).

In Southern Brazil, *Sysirinchium palmifolium* L. (Iridaceae) is a low-growing, abundant and anthophilous grassland flower. It is in the subgenus *Echthronema* which characteristically has yellow flowers that form a funnel-shaped perianth and offers only pollen to foraging insects (Figs. 1a and b). Among the 13 described species occurring in Brazil (Johnston 1938), *S. palmifolium* has the widest distribution, occurring also in Argentina, Uruguay, Paraguay, Bolivia, and Peru. Flowers open for only one day (Heaton & Mathew 1998). At least 19 species of anthophiles, including *Apis mellifera*, were collected at the flowers of *S. palmifolium* at the time of our study (Oliveira F.F. unpubl.).

Having noted at our study site that the flowers of *S. palmifolium* conformed to the general characteristics noted above, and that they were heavily visited by pollen-collecting



FIG. 1. A) Closed flower of *S. palmifolium*; B) Opened flower of *S. palmifolium*; C) and D) Experimental set showing both opened and closed *S. palmifolium* treatments, respectively.

honey bees, only in the mornings, we initiated and completed a short but intensive study with the goal of examining aspects of optimal foraging in pollen-collecting honey bees.

In particular, we tested the following hypotheses: (1) that individual foragers would stay a longer period of time; (2) visit a greater number of flowers; (3) travel greater distances within patches of *S. palmifolium* which were newly opened (early in the morning) or not been previously visited by other honey bees (experimentally closed off) compared to when foraging on patches that were available to pollinators during the whole blooming period (only one day); and (4) to test the hypothesis that any differences in foraging behaviour could be attributed to pollen availability and depletion.

MATERIALS AND METHODS

Study site

Our study site (Fig. 1c) was a grassy area near the buildings of the Pró-Mata Conservation and Research Center from Pontifícia Universidade Católica do Rio Grande do Sul (PMCR-CUCRS) in São Francisco de Paula county, Rio Grande do Sul state, Brazil (29°27'S to 29°35'S and 50°08'W to 50°15'W; altitude 900 m). After initial observations over several days, with intermittent showers, we had two sunny days in which to complete our intensive study (3rd and 4th December 2010).

Behavioural observations and manipulative foraging experiment

Flowers of *S. palmifolium* opened at 8:30 AM and lasted until 1:00 PM, when they closed. Before anthesis, we set up two 2x3 m plots, 5 m apart to assess how honey bees' foraging patterns changed during the day as a result of pollen depletion. Because bees decrease the numbers of flowers they visit with increasing plant density (Beattie 1976; Zimmerman 1981), prior to starting our experiment we manually removed flowers in both plots so that each had approximately 200 and numbered each remaining flower. The positions of the numbered flowers in the plots were

mapped (position located by Cartesian coordinates with accuracy of ± 1 cm).

One plot was left uncovered (designated OP) for the entire blooming period each day. Thus, the bees had complete and natural access to the flowers' pollen resources. At the same time, the second plot (designated CL) was covered with mesh (cheesecloth) to exclude bees until 10:30 AM. During the experiment, we followed the path of individual worker honey bee that entered the OP plot between 08:30 AM and 1:00 PM. Then, at 10:30 AM, the cheesecloth mesh from the CL plot was then removed to allow the honey bees to access the undepleted flowers within. We continued to monitor bee activities in OP and CL simultaneously. We evaluated the following variables: 1) honey bees' foraging path lengths by recording sequentially the individual flowers each visited and referring to the numbered and mapped flowers positions [software tpsDig 2.1 (Rohlf 2006)], 2) time spent (seconds) inside the plot and 3) number of flowers visited by every bee. Whenever a bee left the plot, that visit was considered finished. Bees that visited both plots (i.e. were observed moving from one plot to the other) were excluded from analysis. An additional 1 m² plot was set up to assess flower visiting frequency during the morning of the second experiment day.

Pollen counting

In the first day of the experiment, we arbitrarily took ten *S. palmifolium* flowers from within the plots to assess pollen grains depletion throughout the morning. The anthers of each of the flowers collected were separated and stored in microcentrifuge tubes. In the laboratory, we mixed each anther with a small amount of gelatin coloured with fuchsin at 0.01 % in order to allow the pollen counting (Dafni et al. 2005). The mixture was then spread on a microscope slide, in which the pollen grains were counted using a Tally counter.

Statistical analyses

Bees' foraging patterns throughout *S. palmifolium* anthesis were assessed by Repeated Measures ANOVA with

the data obtained from the OP plot during both mornings. Whenever we found statistical effects, we used planned comparisons to determine which treatment was significantly different from the others, according to our expected hypothesis. We used independent t-tests to compare differences in bees' foraging patterns between OP and CL plots only from 10:30 AM onwards (period when the cheesecloth mesh was removed from CL plot). All variables sampled were used in both kinds of comparisons. The results from anthers of flowers sampled for pollen are presented as statistical means and standard errors of pollen counts at the start and end of the experimental period. We used $\alpha = 0.05$ and verified if the assumptions of every statistical test were met. Whenever necessary, variables were transformed to achieve data normality and homogeneity of variances.

RESULTS

A total of 100 worker honey bees were observed on our plots during the two sampling days: 73 in the OP plot (day 1: n = 30; day 2: n = 43) and 27 were observed in the CL plot (day 1: n = 10; day 2: n = 17). Although the number of bees observed within the OP plot was almost threefold

greater than on the CL plot, it must be remembered that the flowers in the OP plot were available for foraging bees for twice as long as those in the CL plot (eight vs. four hours, respectively). On average and regardless of the kind of plot and time of day, the bees visited 25 ± 20.64 flowers (Mean \pm Standard Deviation) per foraging bout, ranging from one to 120 flowers. The average duration of the foraging bouts inside the plots was 88.95 ± 75.29 sec, ranging from three to 370 sec. The average foraging path length was 7.06 ± 5.84 m, ranging from 0.12 to 30.94 m.

Within the OP plot, from the start of *S. palmifolium* anthesis until bloom ended, honey bees showed marked reductions in the number of flowers they visited (Fig. 2A), time spent during foraging (Fig. 2B), and foraging distances travelled (Fig. 2C). At 8:30 AM, bees visited an average of 27.34 ± 14.16 flowers, spent 91.44 ± 48.26 seconds, and flew 9.76 ± 3.46 meters from the place where they entered the plot to the place they left it. However, at 12:30 PM, on average, those numbers fell to 6.68 ± 3.37 flowers, 41 ± 29.5 seconds, and a distance of only 3.02 ± 1.48 meters. Moreover, the number of flower visitors (Fig. 2D) as well as the availability of pollen grains decreased by the end of

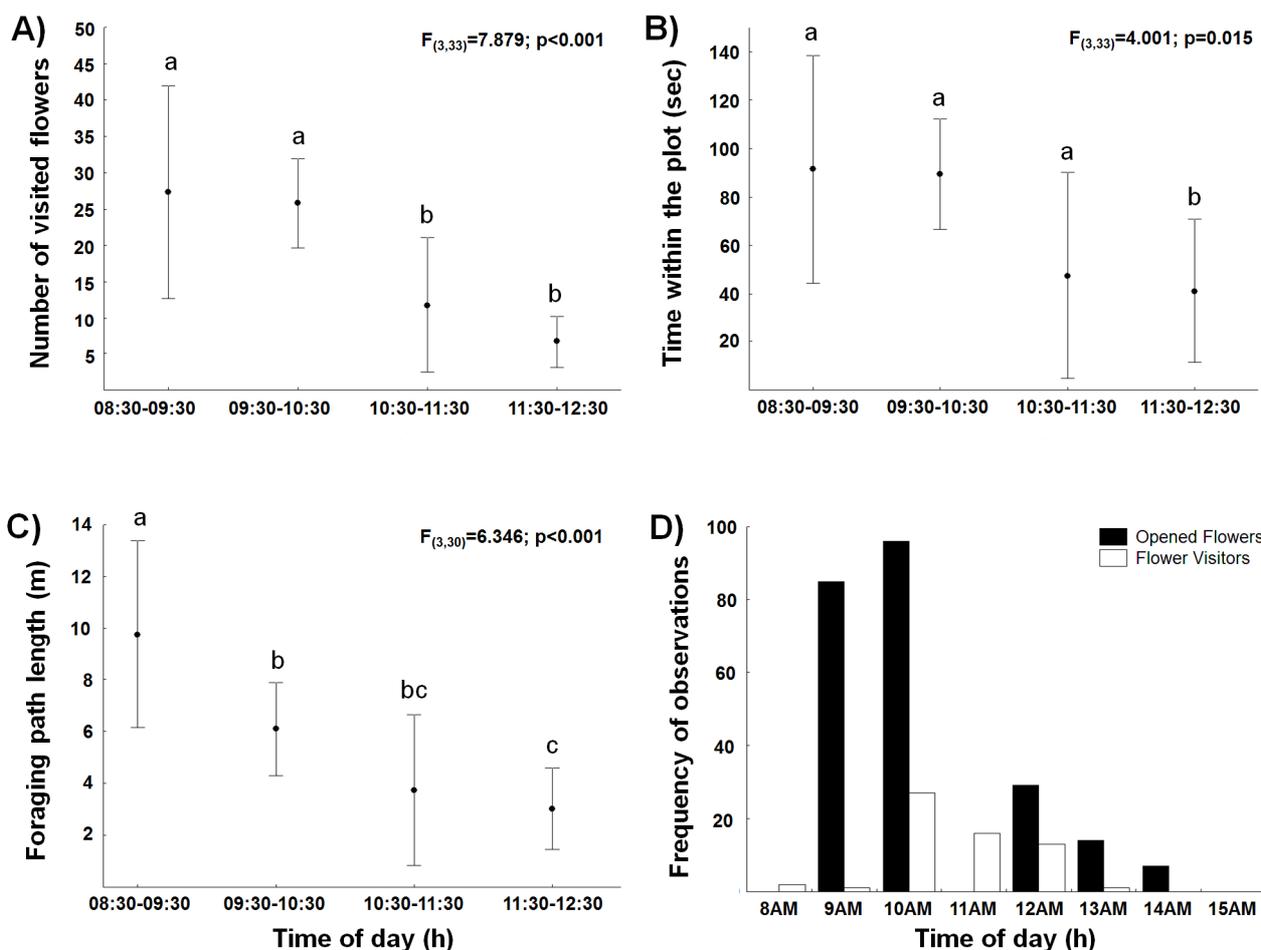


FIG. 2. Daily progression of worker honey bees (*Apis mellifera*) visitation variables on open (remained open throughout the day from 8:30 a.m. on) plots of flowering *S. palmifolium*: A) numbers of flowers visited by worker honey bees, B) times spent by each bee within the plots, C) foraging path distances and D) number of flowers visitors during one day of the sampling period from 10:30 a.m. until the end of anthesis at 12:30 PM. Dots are placed at the statistical mean and bars represent the 95% confidence intervals. Bars with similar letters are not statistically significant ($\alpha \geq 0.05$).

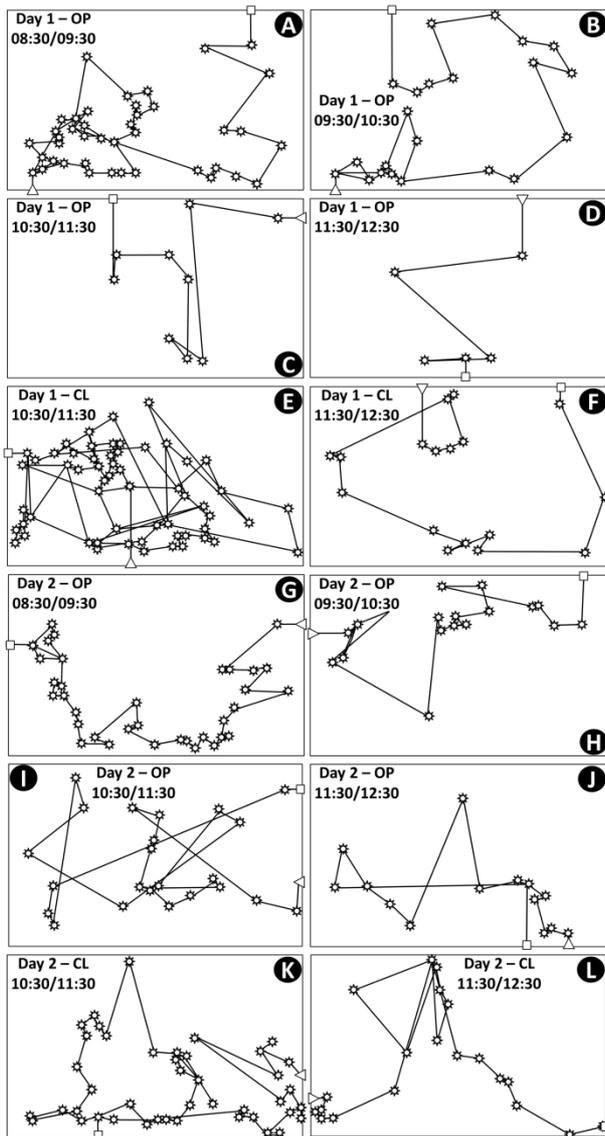


FIG. 3. Longest honey bee (*A. mellifera*) foraging paths observed within the OP and CL plots while foraging on *S. palmifolium*, according to each day and each hour considered in the experiment. Day number and hour are given within each figure. Circles represent flowers' positions in the plot; open triangles represent path's start; open squares represent path's finish in the plot. Note the decline in path distances in the OP plot on day 1 and day 2 from early morning until anthesis (A vs. B vs. C vs. D; then G vs. H vs. I vs. J), and the decline on the closed plot from time of opening until anthesis (E vs. F; then K vs. L). Also note the differences between the lengths of foraging paths at the same times of day but on the different plots (C vs. E and D vs. F for day 1 and I vs. K and J vs. L for day 2).

anthesis from $7,356.6 \pm 829.0$ to 535.3 ± 322.2 grains. Examples of some long foraging paths within each hour, from the OP and CL plots for both sampling days are shown in Figs. 3A-L.

When comparing the foraging patterns in the OP vs. the CL plot, a very clear pattern arises: immediately after the CL plot was uncovered (at 10:30 AM), the quantity of visited flowers, the amount of time spent, and the foraging path length were 3.89, 0.62, and 3.55 times higher than those

values found for the OP plot at the same time (Table 1). Because the assumption of homogeneity of variances was violated in the t-tests, the analyses were made with separated variances (Table 1). The longest foraging paths observed in each period of time (from 8:30 AM until 12:30 PM), in each experiment plot are depicted in Figs. 4A-L. The lengths of the foraging paths and numbers of flowers visited in the newly opened CL plot were similar to those in OP plots early in the morning, but the time the bees spent per flower was greater in the CL plot.

DISCUSSION

In our study, we identified a clear pattern of foraging behaviour changes of honeybee workers collecting pollen from flowers of *S. palmifolium*: bees foraged for shorter distances, shorter time, and at fewer flower as anthesis progressed. Additionally, the honey bees spent less time, visited fewer flowers and flew less over flower patches that were already depleted of pollen when compared to those that were not previously exploited. Previous studies on bees' foraging behaviour already indicated that depletion or decrease of flower resources induces bees to fly over longer distances while searching for foraging resources (Waddington 1983) as our finding clearly demonstrate.

Honey bees are able to remember the period of the day when resources are usually the higher (von Frisch 1963, 1967; Kevan & Menzel 2012), hence their early appearance on the flowers of *S. palmifolium*. They can quickly identify unrewarding patches and might leave them if the amount of resources gained does not compensate for the energy spent collecting it (Zimmerman 1981), as our results show with declining foraging path lengths. We also demonstrate that the foraging bees rapidly adopted foraging strategies similar to those on the undepleted OP patch early in the morning (8:30 AM) on the undepleted CL patch as soon as it was opened at 10:30 AM. Thus, the foraging patterns we recorded accord with the foragers' capacities to respond to rapidly changing mosaics of food resources that vary in profitability daily and seasonally (Butler 1945; Visscher & Seeley 1982; Kevan & Menzel 2012). It is interesting that the bees spent longer while foraging at undepleted flowers on the CL plot once it was opened (138 s) than on OP (early in the day) (90 s).

Perhaps the relatively fewer foragers per flower on the CL plot resulted in the average availability of more pollen per flower. Although floral density can affect foraging patterns (Beattie 1976; Zimmerman 1981), we equalized the abundance of flowers of *S. palmifolium* on our experimental patches. Perhaps, also, as flowers of *S. palmifolium* started to senescent in the early afternoon, the bees switched to foraging at other plants to fulfill daily nutritional needs. Since *A. mellifera* is a pollinator with a wide distribution and is considered to cause important changes on native pollinator communities (Butz Huryn 1997; Paini 2004), we support additional studies evaluating its optimal foraging behaviours in order to better understand how it explores flower resources. Finally, we recognize that our experiment is lacking in replication of plots and days, but we believe its results are interesting and somewhat novel and also confirm

TABLE 1. Comparisons between worker honeybee visitation variables on opened (OP remained open throughout the day from 8:30 AM. on and closed (CP was remained closed until 10:30 AM and then was opened for insect visitation to the flowers) plots of flowering *Sisyrinchium palmifolium* over the two days of the experiments: numbers of flowers visited by worker honey bees, times spent by each bee within the plots, and foraging path distances from 10:30 AM until the end of anthesis at 12:30 PM. *** indicates that the means are statistically significantly different at $\alpha < 0.001$.

Variables	Mean \pm S.D. for OP	Mean \pm S.D. for OP early (8:30 – 10:30 a.m.)	Mean \pm S.D. for CP	t-values for pair- wise tests (d.f.) OP whole day vs. CP	t-values for pair- wise tests (d.f.) OP early vs. CP
Number of flowers visited	9.9 \pm 10.8	29.2 \pm 18.9	38.6 \pm 25.8	-5.376 (34)***	0.775 (29)
Time (sec.) spent per flower	44.5 \pm 51.1	89.9 \pm 49.6	138.5 \pm 98.2	-4.473 (38)***	-2.378 (35)***
Path Length (m) on the patch	3.6 \pm 3.1	8.2 \pm 4.2	11.0 \pm 8.1	-4.486 (33)***	-1.531 (41)

to optimal foraging theory. Therefore, we hope that other researchers will be able to repeat our methods with greater rigour and with more replications in the same or other environments in future studies.

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